

On the Role of the C $\bar{1}$ -Esterase Inhibitor in Cold Urticaria

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Plasma samples from 20 patients with acquired cold urticaria were studied. The C $\bar{1}$ -esterase inhibitor activity was found to be low, but the total C $\bar{1}$ -esterase inhibitor concentration was normal. Prekallikrein, plasmin- α_2 -antiplasmin complex, and kallikrein-like activity were also found to be within normal limits. Cold-promoted activation of coagulation factor VII occurred in 45% of the patient plasmas and resulted in a considerable drop in C $\bar{1}$ -esterase inhibitor activity. *Key words:* Kallikrein-like activity; Prekallikrein; Cold activation of factor VII; Plasmin- α_2 -antiplasmin complex. (Received June 30, 1983.)

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In cold urticaria histamine is released upon cold challenge of the skin and is easily demonstrated in the draining venous blood (1, 2). Pharmacological suppression of histamine release does not inhibit the weal and flare reaction, indicating that inflammatory mediators other than histamine are involved in the pathogenesis of cold urticaria (3). In a previous report describing a delayed urticarial skin response to cold challenge it was suggested that exposure to cold could generate kallikrein-like activity and thereby kinin formation (4).

The C $\bar{1}$ -esterase inhibitor (C $\bar{1}$ INH) is an inhibitor not only of C $\bar{1}$ -esterase but also of kallikrein, plasmin and the Hageman factor (5, 6). Recently, a new method for determining C $\bar{1}$ INH activity in plasma has been described (7). Parallel estimations of C $\bar{1}$ INH activity and C $\bar{1}$ INH antigen can provide useful information about the involvement of the complement system, or the contact activation factors of the coagulation system, in various pathological processes.

In the present investigation of plasma samples from patients with acquired cold urticaria, we were able to demonstrate a decreased activity of C $\bar{1}$ INH with normal concentration of the C $\bar{1}$ INH antigen.

MATERIAL AND METHODS

Patients. We studied 20 patients who attended our clinic because of acquired primary cold urticaria. The mean age was 28 years and the median 22 years (range 7-60 years). The control group consisted of 15 healthy adults (25-35 years old). Plasma samples were collected during the winter season (January-February).

Plasma. Nine volumes of blood obtained by venipuncture were transferred into siliconized Vacutainer tubes (Becton-Dickinson, Mo., USA) containing one volume of 0.13 mol/l citrate. The plasma was collected by centrifugation at room temperature, divided into several sealed plastic vials and stored at -70°C until used.

C $\bar{1}$ -esterase inhibitor (C $\bar{1}$ INH) was purified from freshly frozen human plasma as described earlier (8). The purified protein was found to fulfil the reported specifications (8). Electroimmunoassay (9) with an antiserum raised in a rabbit was used to determine the C $\bar{1}$ INH concentration in plasma. The purified protein served as a standard, using a value of $A_{280\text{ nm}}^{1\text{ g}^1, 1\text{ cm}^1}$ of 0.50 and a molecular weight of 104000 (10). The active concentration of C $\bar{1}$ INH in plasma was estimated according to a recently developed method based on the addition of an excess of purified C $\bar{1}$ s to the sample, and measuring the

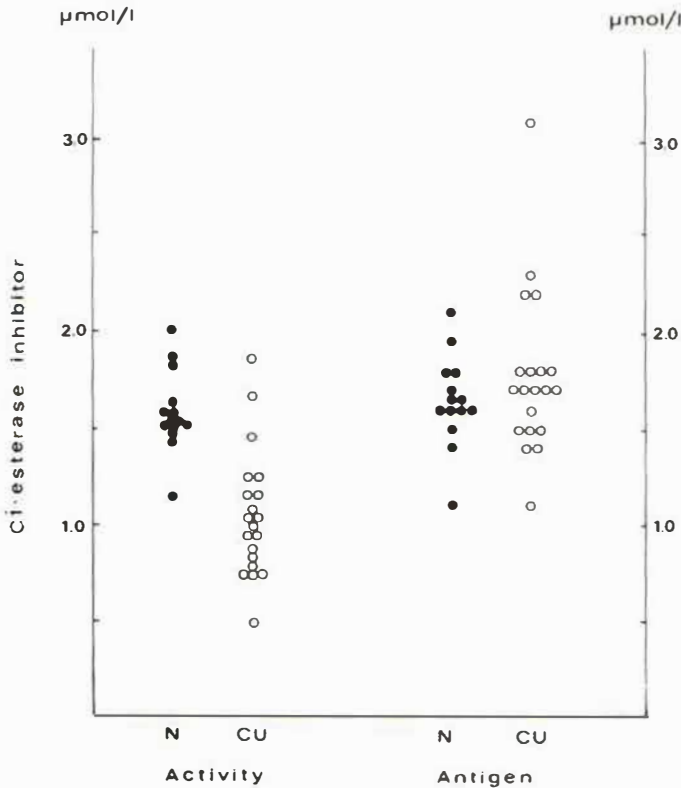


Fig. 1. Plasma concentrations of C1 INH in 20 patients with cold urticaria (CU) and 15 healthy subjects (N) as measured by the activity method (left) and by electroimmunoassay (right).

remaining C \bar{I} s by an amidolytic assay using the chromogenic C \bar{I} s substrate D-Val-Ser-Arg-p-nitroanilide (S-2314) (7). The C \bar{I} s used was prepared as described elsewhere (11).

Prekallikrein was determined using the kit 'Coatest Prekallikrein', a kind gift from Dr Petter Friberger, Kabi Peptide Research, Mölndal, Sweden. The development of the kit has been extensively documented (12).

Kallikrein-like activity was determined by Friberger's method slightly modified (12). Thus, 20 μ l of plasma were added to 0.6 ml of 0.3 mmol/l S-2302 in 0.05 mol/l Tris buffer pH 7.6 containing 0.15 mol/l NaCl and the increase in absorbance at 405 nm (at 37°C) was recorded.

Cold-promoted activation of coagulation factor VII in plasma was carried out by incubating the plasma in sealed plastic tubes in an ice-bath for 24 hours. The prothrombin complex of the cold-incubated samples, and of parallel control samples incubated at 20°C, was measured using Thrombotest (Nyegaard, Oslo, Norway). A shortening of the Thrombotest coagulation time indicates that cold-induced factor VII activation has occurred (13).

Plasmin- α_2 -antiplasmin complex was measured by a recently described radioimmunoassay performed as described (14).

Reagents. The chromogenic tripeptide p-nitroanilide substrates S-2302 and S-2314 were kind gifts from Dr Petter Friberger, Kabi Peptide Research, Mölndal, Sweden.

Statistics. Differences between group means were analyzed using the Student's *t*-test.

RESULTS

C1-esterase inhibitor and prekallikrein concentrations

In Fig. 1 the results from the measurements of plasma concentrations of C1 INH are shown. The concentration of C1 INH in the patient group, as measured by electroimmunoassay, $1.79 \mu\text{mol/l} \pm 0.42 \mu\text{mol/l}$ (SD), is similar to that which was obtained in the control

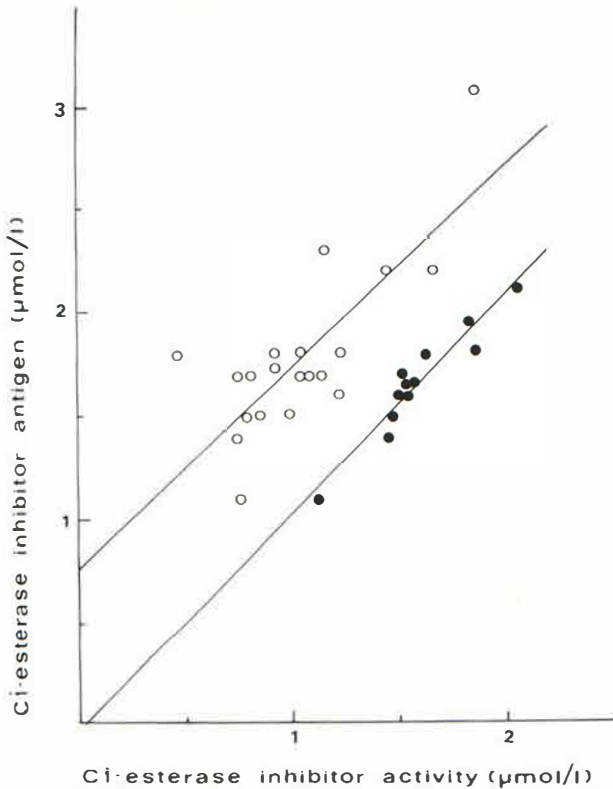


Fig. 2. C1 INH antigen concentrations plotted against active concentrations of C1 INH in 15 healthy subjects (●) and 20 patients with cold urticaria (○). The equation of the lines are $y=1.06x-0.031$ ($r=0.95$) and $y=0.97x+0.76$ ($r=0.77$), respectively.

group, $1.65 \mu\text{mol/l} \pm 0.24 \mu\text{mol/l}$ (SD). However, the active concentration of C1 INH in the patient group is only $1.05 \mu\text{mol/l} \pm 0.33 \mu\text{mol/l}$ (SD) whereas it is $1.59 \mu\text{mol/l} \pm 0.22 \mu\text{mol/l}$ (SD) in the control group. The difference is significant ($p < 0.001$).

From Fig. 1 it can also be calculated that in the patient group the ratio of C1 INH activity to antigen is only $59\% \pm 12\%$ (SD). No patient has more than 77% of the activity that would be expected from the antigen concentration. In contrast the C1 INH in the normal subjects seems to be fully active.

In Fig. 2, the C1 INH concentrations determined by electroimmunoassay are plotted against the values obtained by the activity method. If the data points are analyzed by linear least squares regression, the equation $y=0.97x+0.76$ is obtained ($r=0.77$) for the patient group. This can be compared to $y=1.06x-0.031$ ($r=0.95$) obtained in the control group, and agrees well with the original report describing the activity assay (7).

The results from determinations of prekallikrein were compared to the activity obtained in a pool of the plasma samples of the control group. The patient samples have a prekallikrein concentration of $99\% \pm 10\%$ (SD) of the normal plasma pool.

Kallikrein-like activity

The determinations of kallikrein-like activity produced the results presented in Fig. 3. A large interindividual variation of this parameter has been reported (15) and was also found in the present study. No clearcut difference between the patients and the control group can be seen.

Furthermore, the small values for $\Delta A_{405 \text{ nm}}$ obtained in all plasma samples indicate that

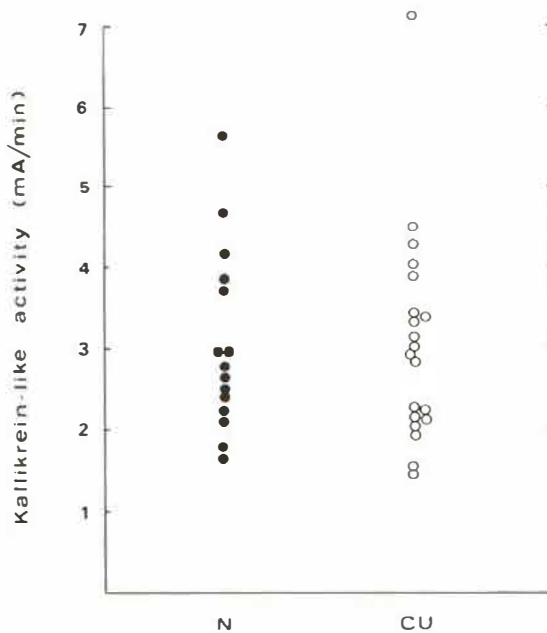


Fig. 3. Kallikrein-like activity of plasma from 15 healthy subjects (N) and 20 patients with cold urticaria (CU), as measured by the amidolytic activity on the chromogenic substrate S-2302.

very small amounts of prekallikrein were activated to kallikrein at sampling, demonstrating the validity of prekallikrein determinations with the activity assay used (see above).

Plasmin- α_2 -antiplasmin complex

Eighteen of the 20 patients did not contain measurable amounts of plasmin- α_2 -antiplasmin complex. This result is similar to the finding in normal individuals (14) indicating the absence of systemic activation of the fibrinolytic system in the cold urticaria patients.

Cold-promoted activation of factor VII

Nine of the 20 patients were found to be 'cold activators' as evidenced by the shortened clotting time after incubation of the plasma samples at 0°C for 24 hours.

The cold-incubated plasma samples were subjected to determination of CI INH activity. Thus, the group which exhibited cold activation ($n=9$) had a mean value of 0.42 ± 0.21 (SD) $\mu\text{mol/l}$ after exposure to cold compared to 1.12 ± 0.30 (SD) $\mu\text{mol/l}$ when left at room temperature (+20°C). The group which did not exhibit any cold activation ($n=11$) had a CI INH activity of 1.10 ± 0.39 (SD) $\mu\text{mol/l}$ after the ice-bath incubation and 1.00 ± 0.35 (SD) $\mu\text{mol/l}$ after incubation at +20°C.

In another experiment, the patient plasmas were made up to a further 3 $\mu\text{mol/l}$ of CI INH by the addition of 1/10 volume of a 30 $\mu\text{mol/l}$ stock solution of the purified protein. No cold activation of factor VII could be elicited among these samples.

There was no correlation between the plasma concentrations of CI INH, prekallikrein, or kallikrein-like activity which could be used to discriminate between the 'cold activators' and the 'non-cold activators'.

DISCUSSION

In patients with chronic urticaria Juhlin & Michaëlsson (16) found that intradermal injection of kallikrein caused an amplified wealing reaction, and therefore suggested that

changes in the concentrations of inhibitors of kallikrein or kallikrein-like enzymes might be involved in the development of urticaria (16). To test this hypothesis the plasma concentrations of protease inhibitors in patients with various forms of urticaria were studied extensively (17, 18). The results show that no major differences can be found that distinguish these patients from the control groups. This could be due to the fact that only the total plasma concentrations of the various protease inhibitors were measured, either by immunochemical techniques, or by relatively unspecific activity methods. However, the methodology developed by Laurell et al. (19) for the study of C \bar{I} -subcomponent complexes reflects the functional state of the proteolytic enzyme systems and their inhibitors rather than the plasma concentrations alone.

We have reported earlier that some patients with chronic urticaria also exhibit a form of cold urticaria, and suggested that activation of kallikrein might play a pathogenetic role in this condition (4). As it has recently become clear that the main inhibitor of kallikrein is the C \bar{I} INH (20), we measured the plasma concentration of C \bar{I} INH in a group of patients with classical acquired cold urticaria, by electroimmunoassay and by a newly developed specific activity assay (7). With this new assay a good 1:1 correlation ($r=0.95$) between C \bar{I} INH activity and antigen was found in plasma of normal individuals, as seen in Figs. 1 and 2. The patients with cold urticaria had a normal concentration of C \bar{I} INH antigen, but a significantly reduced activity (Fig. 1). However, there was still a good 1:1 correlation ($r=0.77$) between the antigen and activity measurements (Fig. 2), indicating that the reason for the low activity could be the presence of inactive forms of the C \bar{I} INH protein.

In attempts to elucidate the reason for the presence of an inactive C \bar{I} INH fraction in patients with cold urticaria, we have performed several types of experiments. The experiments with cold-promoted factor VII activation revealed that 45% of the patients were 'cold activators' as compared to about 15% in the normal population (13). Furthermore, the cold activation resulted in a marked drop in the C \bar{I} INH activity, amounting to about 0.6 $\mu\text{mol/l}$ on average. However, the plasmas still contain measurable activities of C \bar{I} INH (0.4 ± 0.2 $\mu\text{mol/l}$) after cold activation has occurred, in contrast to the findings in another report (21). The plasmas which did not become cold activated had the same C \bar{I} INH activity before and after cold incubation, and this activity was very similar to the activity of the 'cold activators'. This shows that the cold activation is primarily an event due to enzyme activation, irrespective of the plasma C \bar{I} INH activity. However, the enzymes involved must be susceptible to inactivation by C \bar{I} INH since the addition of purified C \bar{I} INH to the plasmas prevented cold activation. This supports the concept that the cold activation mainly depends on the activation of factor XII and prekallikrein (13, 22), since these enzymes are known to be susceptible to the C \bar{I} INH (6).

However, our findings—normal plasma concentrations of prekallikrein and no evidence of increased kallikrein-like activity in the cold urticaria plasmas—indicate that cold-promoted coagulation system activation is not responsible for the lowered C \bar{I} INH activity in cold urticaria plasma. Furthermore, the absence of detectable concentrations of plasmin- α_2 -antiplasmin complex excludes activation of the fibrinolytic system as the reason for the low C \bar{I} INH activity.

If C \bar{I} INH-enzyme complexes are responsible for the lowered C \bar{I} INH activity in cold urticaria, the enzyme in question should thus be the Hageman factor, factor XI or C \bar{I} (6).

An alternative explanation, however, could be the presence in plasma of abnormally functioning C \bar{I} INH molecules. We have recently found that C \bar{I} INH loses its activity in the presence of oxidative agents (T. Nilsson & K. Grankvist, in manuscript) but it is not known whether such reactions also take place *in vivo*.

In conclusion, this study shows that our patients with cold urticaria have decreased activity of C \bar{I} INH but a normal total plasma concentration as measured by electroim-

unoassay. At present, we do not know whether this is due to the presence of C1 INH in complex with proteolytic enzymes, or possibly to the presence of a less reactive fraction of C1 INH molecules.

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